UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON

Department of Medicine
Division of Oncology

December 27, 1974

Harold Varmus, M.D.
Department of Microbiology
University of California
School of Medicine
San Francisco, California

Dear Harold:

We have repeated some of the competition experiments related to the question of the representation in normal cells of the "X piece" sequences of RSV. I enclose a copy of the results of these experiments for your interest. The right-hand panel depicts competition of unlabeled RNA from td Pr-C RNA and parental Pr-C RNA in the hybridization reaction between I^{125} labeled Pr-C RNA and normal chick DNA. Reaction mixtures consisted of .5 mg of fragmented normal chick cell DNA, 2,000 cpm of I^{125} labeled RNA (specific activity 4 x 10^7 cpm/ug), and increasing quantities of unlabeled competitor RNA in 5 x SSC, 50% formamide. Reactions were incubated at 49°C to an uncorrected $\underline{C_o t}$ value of 1.5 x 10^4. About 600 cpm of radioactive RNA acquired RNase resistance (after subtraction of a "zero time" background) in uncompeted control reactions. RNA from both the transformation defective and parental viruses competed virtually completely with the labeled parental 70S RNA. Averaging the 3 points for both types of RNA at the competition plateau there was about an 8 cpm competitive advantage for the parental type RNA (out of a total RNase resistant hybrid of 600 cpm). If one chose to accept this tiny increment in radioactivity as significant, that would suggest that a little more than 1% of the entire RSV genome is represented in the DNA of normal cells, but not in the RNA of the transformation defective mutant.

The right-hand panel depicts, for comparison, the same competition experiment run with RSV induced wing web tumor DNA of the normal DNA. We have repeated the control homologous competition curve with the I^{125} labeled probe, but otherwise the data is the same as was described in the paper we published in J. VIROLOGY. The ordinate is normalized in this graph and represents a 60% uncompeted hybridization at a $\underline{C_o t}$ value of 1.5 x 10^4. About 15% of the hybrids between I^{125} labeled Pr-C RNA on wing web tumor DNA was not competed out by a large excess of unlabeled RNA from the transformation defective mutant. These results are distinctly different than those obtained with

Mailing Address: Division of Oncology, Providence Hospital, 500 17th Avenue, Seattle, Washington 98122
Telephone (206) 322-3140

normal chick DNA. On the basis of this data I have to conclude that at least 90% of the "X piece" sequences which we are measuring in these reactions are not detectable in normal chick embryo DNA. This does not exclude the possibility that 10% or less of the transformation-specific sequences in RSV are still endogenous to the normal chick genome.

I suppose the possibilities that I have suggested before for rationalizing the differences between your data and mine remain viable, and I look forward to hearing more about your results as they progress.

Sincerely yours,

Paul

Paul Neiman, M.D.

PN/lm
Enclosure